



DEPARTMENT OF COMMERCE

National Institute of Standards and Technology

Flow Cytometry Quantitation Consortium

AGENCY: National Institute of Standards and Technology, Department of Commerce.

ACTION: Notice; request for information.

SUMMARY: The National Institute of Standards and Technology (NIST), an agency of the United States Department of Commerce, is establishing the Flow Cytometry Quantitation Consortium and invites organizations to participate in this Consortium. The Consortium will develop reference materials including reference fluorophore solutions and biological reference materials, reference data and reference methods for assigning equivalent number of reference fluorophores (ERF) values and for assessing the associated uncertainties and utilities. Participation in this Consortium is open to all eligible organizations, as described below.

DATES: NIST will accept responses for participation in this Consortium on an ongoing basis. The Consortium's activities will commence on August 15, 2016 ("Commencement Date"). Acceptance of participants into the Consortium after the Commencement Date will depend on the availability of NIST resources.

ADDRESSES: Information in response to this notice and requests for additional information about the Consortium can be directed via mail to the Consortium Manager, Dr. Lili Wang, Biosystems and Biomaterials Division of NIST's Material Measurement Laboratory, 100 Bureau Drive, Gaithersburg, Maryland 20899-8312, or via electronic mail to lili.wang@nist.gov.

FOR FURTHER INFORMATION CONTACT: For further information about partnership opportunities or about the terms and conditions of NIST's Cooperative Research and Development Agreement (CRADA), please contact Honeyeh Zube, CRADA and License Officer, National Institute of Standards and Technology's Technology Partnerships Office, by mail to 100 Bureau

Drive, Mail Stop 2200, Gaithersburg, Maryland 20899, by electronic mail to honeyeh.zube@nist.gov, or by telephone at (301) 975-2209.

SUPPLEMENTARY INFORMATION:

Flow cytometry is a widely used technique for a single cell and particle analysis. It is an essential tool for immunological research, drug and device development, clinical trials, disease diagnosis, and therapy monitoring. The annual expenditure on flow cytometry-related diagnostics is upwards of \$1.2 Billion and growing at more than 10 percent per year, testifying to the economic importance of this technology. The measurements made on the different instrument platforms at different times and locations, however, cannot be compared accurately, which makes diagnostic decisions unreliable and slows down advances in biomedical research. In response to this limitation, NIST and International Society for Advancement of Cytometry (ISAC) have developed a methodology to implement quantitation in flow cytometry. The first step is to calibrate the fluorescence signal from microparticles in terms of a unit of equivalent number of reference fluorophores (ERF) on three laser excitations, 405 nm, 488 nm, and 633 nm. The ERF unit gives the number of reference fluorophores in solution which produce the same fluorescence signal as a single dyed microsphere. The second step uses a biological cell, with known number of specific biomarkers, as a reference material to translate the ERF unit to a unit of antibodies bound per cell (ABC). The ABC unit is most relevant to immunological measurements. To support the calibration of microparticles in terms of ERF, NIST has developed standard reference material (SRM 1934), which includes four solutions of fluorophore: Fluorescein, Nile Red, Coumarin 30 and Allophycocyanin. Microparticles that have been assigned ERF values using SRM 1934 will enable the calibration and characterization of flow cytometers, and the standardization of the fluorescence intensity scale in quantitative ERF units. The results of the collaboration under this Consortium will allow the industry to further research, develop and adopt reference fluorophore solutions for other laser excitations and reference material standards recommended by the expert user community.

NIST is establishing this five-year Consortium to collaborate with manufacturers of microparticles to develop methodologies for assigning ERF values for the microparticles provided to NIST under the scope of the Consortium. The results from this Consortium will also allow NIST to develop the capability that NIST would require to provide a calibration service.

The certificate of analysis for NIST SRM 1934 and NIST's finalized standard operating procedure (SOP) for assigning ERF value will be used for performing the ERF value assignments for participants' microparticles. This SOP includes four steps and is published at J. Res. Natl. Inst. Stand. Technol. 121: 269-286 (2016). As described in the SOP, the ERF value of the major microparticle population is calculated on the basis of the ratio of mean fluorescence intensity values of the major microparticle population to all microparticle populations.

A summary of the ERF value assignments will include ERF values of major microparticle populations, associated combined uncertainties per laser excitation, and reference fluorophore. The combined uncertainty will be derived from all steps of the ERF value assignment, from weighing reference solutions, spectrofluorimeter calibration, CCD response calibration, microparticle concentration measurements by flow cytometer and light obscuration, and measurement of the emission spectrum of microparticles to determine ERF values for major microparticle populations. NIST will also share with each participant any digital emission spectral data of the major microparticle populations. In addition, a participant may request reports for specific ERF value assignments for its microparticles under this Consortium. NIST intends to publish anonymized results of the research under this Consortium. In accordance with 15 USC 3710a(c)(7)(B), NIST will withhold from public disclosure the data that specifically identifies a participant's microparticles for a period of five (5) years from the date any ERF values are generated, or until the participants grants NIST permission to disclose such data. NIST will not require the participants to pay a membership fee to participate in this Consortium. NIST will, however, require participants to

contribute funds to reimburse NIST for the generation of any report requested by a participant for the ERF value assignments of participant's microparticles.

Participation Process: Eligibility will be determined by NIST using the information provided by an organization in response to this notice based on the information requested below.

An organization responding to this notice should provide the following information to NIST's Consortium Manager:

- (1) Type of microparticles: Optimal sizes of microparticles are from 2 to 10 microns. If there are needs of characterization and ERF value assignment to other size particles (<2 microns or >10 microns), the present standard operating procedure can be modified to accommodate the requests.
- (2) Type of Instrument: The Consortium is to assign ERF values for microparticles used primarily for flow cytometers. Any information about other instruments used by the organization is helpful to ensure that there is diversity in participants. For example, please indicate if the microparticles are used by the organization with fluorescence microscopes and spectrophotometers/spectrofluorimeters.
- (3) Experience in production and characterization of microparticles, antibodies, and biological cells, and analysis of large data sets.

A responding organization should not include any business proprietary information in its response to this request for information. NIST will not treat any information provided in response to this request as proprietary information. NIST will notify each organization of its eligibility. In order to participate in this Consortium, each eligible organization must sign a Cooperative Research and Development Agreement (CRADA) for this Consortium. All participants to this Consortium will be bound by the same terms and conditions.

Kent Rochford,

Associate Director for Laboratory Programs.

